MERIT RESEARCH JOURNALS
www.meritresearchjournals.org

Merit Research Journal of Food Science and Technology (ISSN: 2354-2527) Vol. 2(1) pp. 001-007, January, 2014
Available online http://www.meritresearchjournals.org/fst/index.htm

Full Length Research Paper

Isolation and characterization of heterotrophic micro-organisms and dominant Lactic Acid Bacteria (LAB) from different brands of yoghurt and ice cream

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Abstract

Different brands of yoghurt and ice cream were analyzed microbiologically. Routine laboratory procedure was adopted using standard methods and microbiological media. High microbial counts indicate gross contamination with both pathogenic bacteria and fungi. *Staphylococcus aureus* and *Bacillus cereus* produce toxins of medical and health importance. Mycotoxin producing moulds such as *Aspergillus* and *Penicillium* species were also isolated. Food borne illnesses associated with these bacteria and fungi has been documented. Lactic acid bacteria such as *Streptococcus thermophilus* and *Lactobacillus bulgaricus* dominated in the products analyzed. Other lactic acid bacteria with probiotic potential were also isolated. Although yoghurt and ice cream rich are in food nutrients and live organisms of health benefits, if inadequately processed and consumed will be detrimental to human health. To ensure high quality, safe and acceptable products, good manufacturing practices that highlight the critical control points should be considered.

Keywords: Yoghurt, Ice cream, Microbial quality, Dominant LAB.

INTRODUCTION

Lactic Acid Bacteria (LAB) are indispensable in fermentation processes. LAB were first isolated from milk (Carr *et al*., 2002) and have since been found in such foods as fermented products as meat, milk products, vegetables, beverages and bakery products (Aukrust and Blom, 1992; Caplice and Fitzgerald, 1999; Harris *et al*., 1992; Gobbetti and Corsetti, 1997; Jay, 2000; Savadogo *et al*., 2006; Liu, 2003; Lonvaud-Funel, 2001). LABS occur naturally in fermented food (Caplice and Fitzgerald, 1999; Abdelbasset and Djamila, 2008; Oranusi *et al*., 2011) and have been detected in soil, water, manure and sewage (Holzaphfel *et al*., 2001). LAB exist in human (Elliot *et al*., 1991; Martin *et al*., 2003; Ocana *et al*., 1999; Reid, 2001; Schrezenmeir and de Vrese, 2001) and in animals (Fujisawa and Mitsuoka, 1996).

These autochthnous bacteria interact with the diet and the host, contributing to protection against intestinal pathogens through colonization, resistance and providing nutritional and colonic health benefit via their metabolic activities (Guarner and Malagelada, 2003; Sleator and Hill, 2008; Sleator, 2010).

LAB is regarded as a major group of probiotic bacteria (Schrezenmeir and de Vrese, 2001). The probiotic concept has been defined by Fuller (1989) to mean "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance".

Several lactobacilli, lactococci and bifidobacteria are held to the health - benefiting bacteria (Rolfe, 2000; Tuohy *et al*., 2003). Lactic acid bacteria along with other gut microbiota ferment various substrates like lactose, biogenic amines and allergenic compounds into short chain fatty acids and other organic acids and gases (Gibson and Fuller, 2000; Jay, 2000). LAB synthesizes enzymes, vitamins, antioxidants and bacteriocins (Knorr, 1998). With these properties, intestinal LAB constitutes an important mechanism for the metabolism and detoxification of foreign substances entering the body (Salminen, 1998). The health-promoting effects of LAB are strain specific and result in different mechanisms to produce beneficial health impacts including control of intestinal disorder and the production of antimicrobial substances such as lactic acid, H_2O_2, diacetyl and bacteriocins (Hoover, 2000; Grangette *et al*., 2001; Perdigon *et al*., 1999; Cross, 2002).

In Nigeria, yoghurt and ice-cream are among the most popular and affordable dairy products. Some consume it to quench taste on a hot sunny day; others used it as dessert. Recently, the Nigerian market and departmental shops has been flooded with so many brands of these products manufactured in the country. The quality and standard of some are in doubt and questionable. This curiosity prompted the investigation into the dominant lactic acid bacteria species as well as the heterotrophic bacteria present in the products.

MATERIALS AND METHODS

Description and collection of samples

Twenty brands of yoghurt and five brands of ice cream were obtained from retail outlets, departmental stores, fast food outlets and markets in Nigeria. All the products were well packaged and have Nigerian identity with NAFDAC approved numbers.

Microbiological analysis

Ten milliliters of thawed samples were dispersed in 90ml of sterile distilled water to obtained 10^{-1} dilution. Further dilution was made by transferring 1ml into 9ml distilled water until 10^{7} dilutions was obtained. Aliquot portion (0.1ml) of 10^{7} and 10^{6} dilution was inoculated surface dried nutrient and MacConkey agar respectively. The same quantity (0.1ml) was transferred from 10^{5} and 10^{4} into LAB media, Brain Heart Infusion Agar and De Mann Rogosa and Sharpe Agar (BHIA and MRS) and Potato Dextrose Agar respectively. Inocula were spread evenly and plates incubated at recommended temperature and time (Beishir, 1987; Cheesbrough, 2000).

Colony counts

Microbial counts were done with digital Gallenkamp colony counter. Total count was expressed as colony forming units per milliliter (CFU/ml).

Characterization and identification of isolates

Bacteria and yeasts isolates was identified by the methods described by Beishir (1987), Harrigan and McCance (1990) and Cheesbrough (2000). Arrangement of mycelia, sporulation and pigmentation were the criteria used for the identification of moulds (Frazier and Westhoff, 1987; Harrigan and McCance, 1990; Abbey, 2007). Standard manuals by Buchanan and Gibbon (1974) and Barnett and Hunter (1987) were used to identify bacteria and fungi respectively.
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RESULTS

The total bacterial count for the yoghurt ranges from 1.4×10^{8} to 2.04×10^{8} on NA, 1.0×10^{6} to 1.48×10^{7} on MCA, 1.9×10^{6} to 1.11×10^{7} on MRS, 1.0×10^{5} to 2.01×10^{7} on BHI and 1.0×10^{5} to 9.2×10^{7} on PDA medium respectively. The total bacterial count for the ice cream ranges from 5×10^{6} to 8.1×10^{7} on NA, 1.0×10^{6} to 2.1×10^{6} on MCA, 1.0×10^{5} to 7.0×10^{5} on PDA, 5×10^{5} to 5.3×10^{6} on BHI and 1.4×10^{7} to 3.9×10^{7} on MRS medium respectively (Table 1). Heterotrophic bacteria count is higher than the lactic acid bacteria for all the samples analysed. Fungal counts are comparable to the LAB.

Tables 2, 3 and 4 shows the general characteristics and identities of the heterotrophic bacteria, lactic acid bacteria and fungi respectively isolated from the samples. Heterotrophic bacteria isolated are species of *Bacillus, Micrococcus, Enterococcus*, and *Staphylococcus. Lactobacillus, Bacillus* and *Streptococcus* species were isolated among the lactic acid bacteria. *Penicillium notatum, Rhizopus stolonifer, Aspergillus, Mucor* and *Saccharomyces* species were fungi isolated. The dominant lactic acid bacteria isolated from the yoghurt and ice cream are *Streptococcus thermophilus, Lactobacillus casei, Lactobacillus bulgaricus, Lactobacillus lactis, Lactobacillus acidophilus* and *Bacillus subtilis* as shown in Table 5.

Table 1. Microbial Counts (cfu/ml) of Samples yoghurt

Sample code	Total counts on NA	Total counts on MCA	Total counts on PDA	Total counts on BHI	Total counts on MRS
HOLA (A)	3.1×10^{7}	2.1×10^{5}	8.1×10^{6}	5.0×10^{5}	2.0×10^{6}
HOLC (B)	5.0×10^{7}	4.5×10^{5}	3.2×10^{6}	1.7×10^{5}	3.8×10^{6}
HOLE (C)	7.0×10^{6}	1.1×10^{5}	1.9×10^{6}	2.0×10^{5}	1.9×10^{6}
DAVA(D)	8.0×10^{6}	7.1×10^{5}	2.8×10^{6}	1.0×10^{5}	2.4×10^{6}
BOYA (E)	4.0×10^{6}	2.8×10^{5}	1.4×10^{6}	1.0×10^{5}	3.2×10^{6}
HOLB(F)	2.0×10^{7}	1.7×10^{5}	1.0×10^{6}	1.69×10^{7}	4.9×10^{6}
HOLD (G)	1.4×10^{8}	1.9×10^{5}	2.8×10^{6}	2.01×10^{7}	1.00×10^{7}
HOLF (H)	1.69×10^{9}	1.01×10^{6}	9.2×10^{7}	1.52×10^{7}	1.11×10^{7}
DAVB (I)	1.09×10^{9}	1.21×10^{6}	4.9×10^{7}	4.0×10^{6}	3.1×10^{6}
BOYB (J)	1.21×10^{9}	9.2×10^{5}	7.1×10^{7}	2.6×10^{7}	2.8×10^{6}
KABS (K)	2.5×10^{7}	2.2×10^{6}	1.9×10^{6}	4.0×10^{6}	7.1×10^{7}
SUPA (L)	3.2×10^{7}	6.0×10^{6}	3.6×10^{6}	2.9×10^{6}	4.9×10^{7}
OLEK (M)	2.6×10^{8}	4.1×10^{6}	2.0×10^{6}	6×10^{5}	2.8×10^{7}
FANS (N)	4.0×10^{7}	4.1×10^{6}	3.0×10^{6}	2.80×10^{7}	5.3×10^{7}
JIRE (O)	4.2×10^{7}	1.48×10^{7}	1.8×10^{6}	2.0×10^{6}	1.9×10^{7}
DEHM (P)	2.04×10^{7}	1.7×10^{6}	6.0×10^{5}	2.0×10^{6}	5.0×10^{6}
UMMZ (Q)	7.5×10^{7}	2.1×10^{6}	4.0×10^{5}	1.01×10^{7}	4.1×10^{6}
FARM (R)	4.2×10^{7}	1.0×10^{6}	7.0×10^{5}	2.5×10^{6}	5.4×10^{6}
HOLP (S)	1.5×10^{7}	1.1×10^{6}	2.0×10^{5}	2.0×10^{6}	3.1×10^{6}
DICT (T)	4.7×10^{7}	2.1×10^{6}	1.0×10^{5}	2.1×10^{6}	4.6×10^{6}
Ice Cream					
CRUN (1)	8×10^{7}	1.7×10^{6}	6.0×10^{5}	1.3×10^{6}	1.4×10^{7}
BAMB (2)	8.1×10^{7}	2.1×10^{6}	4.0×10^{5}	5.3×10^{6}	2.9×10^{7}
DESM (3)	2.5×10^{7}	1.0×10^{6}	7.0×10^{5}	5×10^{5}	3.5×10^{7}
BIGG (4)	5×10^{6}	1.1×10^{6}	2.0×10^{5}	1.7×10^{6}	2.1×10^{7}
RENN (5)	4.3×10^{7}	2.1×10^{6}	1.0×10^{5}	1.3×10^{6}	3.9×10^{7}

Table 2. Characteristics of bacteria isolated from yoghurt and ice cream on bacteriological medium

	Microscopic Characteristics															
Colonial Characteristics	Grm rxn	Mot	Spo	Cap	Cat	Oxi	Coag	In	MR	VP	Cit	G	L	S	M	Mn	Identity of Isolates
Dull and dry irregular flat cream colonies	+R	+	+	-	+	-	-	-	-	+	+	+	$+^{s}$	-	-	-	*Bacillus* sp.

Table 2. Continue

Slimy and mucoid cream colonies	+R	+	+	-	+	-	-	-	-	+	+	+	$+^s$	-	-	+	*Bacillus subtilis*
Small smooth and shiny low convex yellow colonies	+S	-	-	-	+	-	-	-	+	-	+	-	-	-	-	-	*Micrococcus luteus*
Smooth and circular orange colonies	+S	-	-	-	-	-	-	-	+	-	+	+	-	+	-	-	*Micrococcus roseus*
Small circular and shiny cream colonies	+S	-	-	-	-	-	-	-	+	-	+	+	+	+	-	+	*Enterococcus faecalis*
Golden yellow smooth and shiny colonies	+S	-	-	-	+	-	+	-	-	+	-	+	+	+	+	+	*Staphylococcus aureus*

Mot, Motility; Spo; Spore; Cap, Capsule; Cat; Catalase; Ar, Arabinose; Fr, Fructose, G, Glucose; L, Lactose; M, Maltose; S, Sucrose; Xyl, Xylose; d, delay; $+^s$, Slow reaction.

Table 3. Characteristics of LAB isolated from yoghurt and ice-cream sample

Colonial Characteristics/ BHI A MRS	Microscopic Characteristics				Biochemical Characteristics								Identity of Isolates
	Gram rxn	Mot	Spo	Cap	Cat	Ar	Fr	G	L	M	S	Xyl	
Cream mucoid and slimy colonies	+R	+	+	-	+	+	+	+	-	-	-	+	*Bacillus subtilis*
Colonies usually rough, irregular shape with radiate projection	+R	-	-	-	-	-	+	+	+	+	+	-	*Lactobacillus acidophilus*
Colonies are light orange, moist and shiny with round edges	+R	-	-	-	-	-	+	+	+	-	-	-	*Lactobacillus bulgaricus*
Colonies normally rough and white to light grey	+R	-	-	-	-	-	+	+	+	+	+	-	*Lactobacillus lactis*
Colonies smooth, diamond shaped white to very light yellow colour	+R	-	-	-	-	-	+	+	$+^s$	d	d	-	*Lactobacillus casei*
Small circular and shiny deep yellow colonies	+S	-	-	-	-	+	+	+	-	-	+	+	*Streptococcus thermophilus*

Table 4. Characteristics of fungi isolated from yoghurt and ice cream on PDA

Colonial characteristics	Microscopic characteristics	Identity of isolates
Green powdery spores enclosed in a short white mycelium radiating outwards	Hyphae is septate. Conidia arranged like a mop on a sterigma	*Penicillum notatum*
Black spores at the apex attached to short white mycelia	Hyphae is septate. Coniolia club-like attached to a vesicle via a sterigma	*Aspergillus* sp.

Table 4. Continue

Tall white filamentous hyphae covering the entire culture plate	Non-septate hyphae. Sporangiospores enclosed in a sporangium at the base of the sporangiophere.	*Rhizopus stolonifer*
Cream butyrous circular colonies dull and dry	Large oval and spherical gram positive budding cells	*Saccharomyces cerevisiae*
Short white mycellium spreading across the culture plates	Non-septate hyphae, but sporangiophores are septate. Spores are scattered	*Mucor* sp.
Small circular and shiny low convex cream colonies	Gram positive ellipsoidal shaped cells with small projections (buds) attached to bigger cells	*Saccharomyces ellipsoideus*

Table 5. Dominant LAB isolated from yoghurt and ice-cream

Samples code yoghurt	**Lactic Acid Bacteria (LAB)**
HOLA (A)	*L. bulgaricus; S. thermophilus; L. acidophilus*
HOLC (B)	*L. bulgaricus; S. thermophilus*
HCLE (C)	*L. casei; L. bulgaricus; S. thermophilus*
DAVA (D)	*L. bulgaricus; S. thermophilus; L. acidophilus*
BOYA (E)	*S. thermophilus; L. bulgaricus*
HOLB (F)	*S. thermophilus; L. bulgaricus*
HOLD (G)	*L. bulgaricus; L. acidophilus; S. thermophilus*
HOLF (H)	*L. bulgaricus; L. acidophilus; S. thermophilus*
DAVB (I)	*L. bulgaricus; S. thermophilus*
BOYB (J)	*S. thermophilus; L. bulgaricus*
KABS (K)	*L. casei; B. subtilis*
SUPA (L)	*L. acidophilus; L. casei*
OLEK (M)	*L. lactis; L. bulgaricus*
FANS (N)	*L. bulgaricus; L. casei; B. subtilis*
JIRE (O)	*L. lactis; L. acidophilus*
DEHM (P)	*L. bulgaricus; S. thermophilus; L. acidophilus*
UMMZ (Q)	*L. bulgaricus; S. thermophilus;*
FARM (R)	*L. bulgaricus; S. thermophilus; L. acidophilus*
HOLP (S)	*S. thermophilus; L. acidophilus*
DICT (T)	*L. bulgaricus; S. thermophilus*
Ice Cream	
CRUN (1)	*L. lactis; L. casei; B. subtilis*
BAMB (2)	*L. bulgaricus; L. lactis*
DESM (3)	*L. lactis; L. acidophilus; B. subtilis*
BIGG (4)	*L. lactis; L. casei*
RENN (5)	*L. acidophilus; B. subtilis; L. lactis*

DISCUSSION

Ice cream and yoghurt are fermented dairy products whose major material (milk) is obtained from animal, especially cow. High incidence of heterotrophic bacteria and fungi recorded in this study suggest gross contamination during or after production. The presence of some pathogenic bacteria and fungi such as *Staphylococcus aureus, Bacillus, cereus, Enterococcus faecalis, Aspergillus flavus, Rhizopus stolonifer, Penicillium notatum* and *Mucor* species potent serious hazard to the final consumers. *Staphylococcus aureus* and *Bacillus cereus* produce toxins if when ingested cause food borne infection and intoxication (Frazier and Westhoff, 1987). Fungi are generally regarded as spoilage organisms of foodstuffs (Mossel *et al*., 1995; Effiuvwevwere, 2007); their occurrence is also recognized as a source of potential health hazard to humans and animals globally. *Aspergillus, Mucor, Penicillium* and *Rhizopus* produce mycotoxins capable of inducing mycotoxicoses following ingestion and inhalation (Nijis *et al*., 1997; Smith *et al*., 1994, Effiuvwevwere, 2007).

Streptococcus thermophilus and *Lactobacillus bulgaricus* are used as starter cultures in the production of yoghurt in the dairy industries. They are either added as pure cultures or in a rare case back slopped from previous fermented yoghurt. The role played by other lactic bacteria in yoghurt production is not clearly understood, although their probiotic potentials had been discussed (Collins *et al*., 1998; Metchnikoff, 1908; Schrezenmeir and de Vrese, 2001).

Raw cow milk had been reported to host microorganisms associated with food borne infection and intoxication (Frazier and Weshoff, 1978) including *Streptococcus agalactiae,* the causative agent of mastitis in cow and cattle. Inadequate sanitation of utensils and equipment used in the processing of dairy products had also been incriminated in microbial contamination (Frazier and Westhoff, 1978).

Good manufacturing practices including strict compliance to proper sanitary conditions and pasteurization of raw milk could be a *panacea* to producing yoghurt and ice cream of a wholesome quality. In addition, the use of pure viable starter cultures will minimally reduce contaminants.

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